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Immunocytochemistry Followed by Electron Microscopy

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Antifade (1.4-phenylene-diamine)
Bovine Serum Albumin (BSA)
Boehringer Mannheim Biochemicals (BMB), Cat. 100 350
Gridded coverslips
Bellco, Cat. 1916-92525
DAPI
BMB, Cat. 236 276
EGTA
Sigma, Cat. E3889
Glutaraldehyde, 25% EM grade
Polysciences, Inc., Cat. 01909
Goat anti-rabbit-TRITC (2° Ab)
Sigma, Cat. T-5268
Normal Goat Serum (NGS)
Sigma, Cat. G6767
Magnesium chloride ($MgCl_2$), 2 M
Quality Biological, Inc., Cat. 340-034-060
1X Phosphate Buffered Saline (PBS), pH 7.4
Gibco/BRL, Cat. 10010-023
PIPES
Sigma, Cat. P9291
Rabbit polyclonal antibodies (1° Ab)
Specific for desired protein
Sodium borohydride ($NaBH_4$)
Sigma, Cat. S9125
Sodium chloride (NaCl)
Mallinckrodt, Cat. 7581
Sucrose
Sigma, Cat. S7903
Triton X-100
Calbiochem, Cat. 648462

Preparation

Cytoskeleton (CSK) Buffer

PIPES	1.512 g	f.c. [10mM]
Sucrose	51.35 g	f.c. [300mM]
NaCl	2.923 g	f.c. [100mM]
0.5 M EGTA	0.19 g	f.c. [1mM]
2M $MgCl_2$	0.75 ml	f.c. [3mM]

*Bring to 500 ml with sterile distilled water

0.1% Sodium Borohydride solution
Prepared fresh 1mg/ml in 1X PBS

Blocking Solution (5% NGS/ 5% BSA/1X PBS)

NGS	500 µl
BSA	0.5 g
1X PBS	10 ml

Store at 4°C

Antibody Solution (1% NGS/1% BSA/1X PBS)

Blocking Solution I	200 µl
1X PBS	800 µl

DAPI (stock solution)

DAPI	2 mg
dH_2O	10 ml

Aliquot and store at -80ºC

DAPI (staining solution)

DAPI stock solution	40 µl
2X SSC	100 ml

Antifade (1,4-phenylene-diamine)
See Antifade preparation procedure in CGH Protocols

Procedure

1. Grow adherent cells on gridded coverslips or cytospin suspension cells onto poly-L-lysine coated gridded coverslips.

2. Fix cells in CSK Buffer + 0.1% Triton-X100 for 30 sec.

3. Fix cells in CSK Buffer + 0.1% Triton-X100 + 1% glutaraldehyde for 2 min.

4. Fix cells in CSK Buffer + 1% glutaraldehyde for 10 min.

5. Wash 2 x 15 min with fresh 0.1% sodium borohydride solution.

6. Block coverslips with 25µl blocking solution in hybridization chamber 30 min at 37˚C.

7. Incubate with rabbit polyclonal 1˚ Ab in 25 µl antibody solution in hybridization chamber at 37˚C for 60 min.

8. Wash 3 x 5 min with 1X PBS at RT.

9. Incubate with 2˚ Ab [goat anti-rabbit-TRITC; 1:200 in 25µl antibody solution] in hybridization chamber at 37˚C for 60 min.

10. Wash 3 x 5 min with 1X PBS at RT.

11. Stain for 2 min with DAPI.

12. Wash in 1X PBS for 10 min, shaking.

13. Mount coverslip with 10 µl antifade on microscope slide.

14. Image cells of interest.

15. Send coverslips in 1X PBS for EM analysis.